Reference: *Biol. Bull.*, **140**: 376–388. (June, 1971)

COMPARISON OF CHROMATOPHOROTROPINS FROM THE HORSESHOE CRAB *LIMULUS POLYPHEMUS*, AND THE FIDDLER CRAB, *UCA PUGILATOR*[1]

MILTON FINGERMAN, CLELMER K. BARTELL AND ROBERT A. KRASNOW

Department of Biology, Tulane University, New Orleans, Louisiana 70118, Department of Biology, Louisiana State University, New Orleans, Louisiana 70122, and Marine Biological Laboratory, Woods Hole, Massachusetts 02543

Chromatophore responses of fiddler crabs have been utilized to study the chromatophorotropins that these crabs produce themselves and, in addition, to assay for chromatophorotropic substances from other species of invertebrates. Brown and Cunningham (1941) found that extracts of the central nervous system (CNS) of the horseshoe crab, *Limulus polyphemus*, cause dispersion of the pigment in the melanophores of the fiddler crab, *Uca pugnax*, and concentration of the pigment in its leucophores. The melanin-dispersing and white pigment-concentrating responses evoked by the seven portions of the CNS tested paralleled each other so closely that Brown and Cunningham suggested both effects may have been caused by a single substance. More recently, Rao and Fingerman (1970a) investigated the chromatophorotropic activity of extracts of radial nerves from the sea star, *Asterias amurensis*, and found that after gel filtration certain fractions which caused melanin dispersion in the fiddler crab, *Uca pugilator*, also evoked pigment concentration in the leucophores and erythrophores. Extracts of horseshoe crab CNS have not been tested on erythrophores of fiddler crabs. On the other hand, extracts of the eyestalks of the fiddler crab, *Uca pugilator*, have been clearly shown to contain substances that will cause dispersion of the pigment in its melanophores, leucophores and erythrophores and concentration of the pigment in its leucophores and erythrophores (Carlson, 1935; Sandeen, 1950; Brown, 1950; Rao, Fingerman and Bartell, 1967). Differential solubility analyses and gel filtration studies of chromatophorotropins from the eyestalks of the fiddler crab, *Uca pugilator*, indicate that each chromatophorotropin has either a pigment-dispersing or pigment-concentrating action but not both (Rao, Fingerman and Bartell, 1967; Fingerman and Couch, 1967; Fingerman and Rao, 1969a, 1969b). The present investigation was undertaken (a) to determine whether the active material in the CNS of the horseshoe crab has the same elution pattern from Bio-Gel P-6 and evokes the same chromatophoric responses in the fiddler crab as did that from the radial nerves of the sea star and (b) to compare some of the properties of chromatophorotropins from the CNS of the horseshoe crab and the eyestalks of the fiddler crab.

Materials and Methods

The horseshoe crabs, *Limulus polyphemus*, were collected in the vicinity of Woods Hole, Massachusetts, by members of the Supply Department of the Marine Biological Laboratory. We wish to express our appreciation for their efforts. The fiddler crabs, *Uca pugilator*, were obtained from Panacea, Florida. The total

[1] This investigation was supported by Grant GB-7595X from the National Science Foundation.

length of the horseshoe crabs used in this investigation was 105–125 mm; carapace width of the fiddler crabs was 16–19 mm.

The extracts were assayed on the melanophores, erythrophores and leucophores of the fiddler crab. The melanin and red pigment of eyestalkless fiddler crabs was maximally concentrated; the red pigment of intact fiddler crabs in black containers is maximally dispersed; and the white pigment of intact Panacea fiddler crabs in a black container is maximally concentrated. The white pigment of eyestalkless Panacea fiddler crabs is found in all stages ranging from maximal concentration to maximal dispersion. Eyestalkless individuals whose white pigment was maximally dispersed were selected for use in these experiments. Therefore, by making use of intact and eyestalkless fiddler crabs assays could be made for melanin-dispersing, red pigment-dispersing, white pigment-dispersing, red pigment-concentrating and white pigment-concentrating substances. The Hogben and Slome (1931) method was used to stage the chromatophores on the anteroventral surface of the second or third walking leg. According to this system stage 1 represents maximal concentration of the pigment, stage 5 maximal dispersion and stages 2, 3 and 4 the intermediate conditions. These chromatophore stages were used to calculate Standard Integrated Responses (SIR) to extracts as defined by Fingerman, Rao and Bartell (1967). The SIR is a measure of both the amplitude and duration of the response.

Extracts of the tissues to be assayed, the CNS of the horseshoe crab and the eyestalks of the fiddler crab, were prepared in several different ways depending upon the aim of the experiment. The particular method used will be presented in the appropriate place below as the experiments are described. In those gel filtration experiments where distilled water was used to elute the active material from the column, each fraction was mixed with an appropriate volume of 400% physiological saline (Pantin, 1934) to provide an extract that was isosmotic with the blood of the fiddler crab. Otherwise 100% saline was used. The injected dose was 0.05 ml per crab. Control crabs received 100% saline alone. Each fraction obtained from the gel chromatography experiments was assayed on three crabs; all other extracts were assayed on five crabs.

Experiments and Results

Comparison of the chromatophorotropic activity of the ethanol-soluble fraction, the 95% methanol:chloroform (2:1)-soluble fraction and a saline extract of the central nervous system of the horseshoe crab

The first objective of this series of experiments was to assay extracts of the horseshoe crab CNS prepared directly in saline on the melanophores and leucophores of a fiddler crab as did Brown and Cunningham (1941), but *Uca pugilator* would be used instead of the species, *Uca pugnax*, they used. In addition the effect of such an extract on the erythrophores of a fiddler crab would also be determined for the first time. Secondly, it has been demonstrated that the ethanol-soluble fraction (Rao, Bartell and Fingerman, 1967) and the methanol:chloroform-soluble fraction (Bartell, Rao and Fingerman, 1971) of the eyestalks of the fiddler crab, *Uca pugilator*, prepared in concentrations higher than 0.3 eyestalk equivalent per dose evoke much stronger melanin-dispersing responses than do saline extracts

but below this concentration the differences among the responses to such extracts are slight. However, a high activity ethanol-soluble fraction is not obtained when freeze-dried eyestalks are used (Rao, Bartell and Fingerman, 1968). For comparative purposes melanin-dispersing and white pigment-concentrating responses

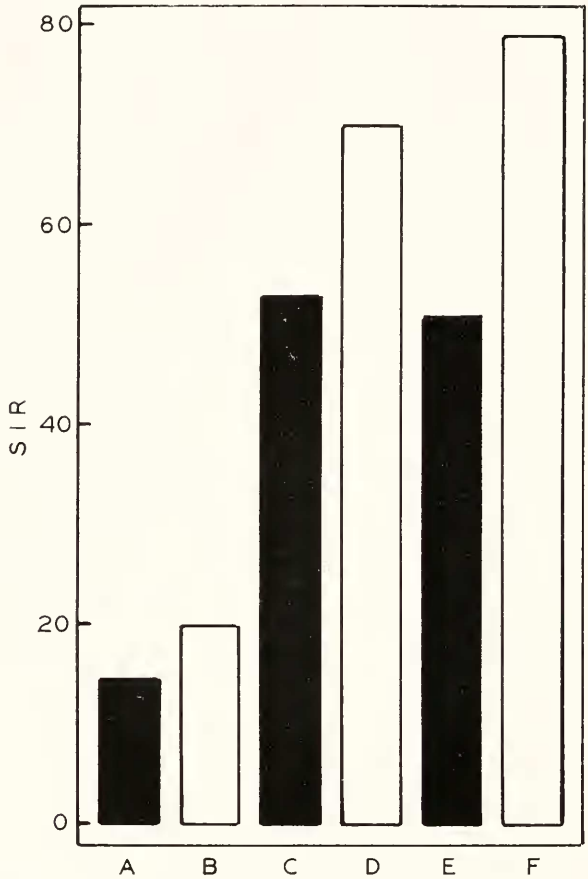

FIGURE 1. The Standard Integrated Responses (SIR) of eyestalkless fiddler crabs to extracts of the central nervous system of the horseshoe crab; solid bars, melanin-dispersing responses; empty bars, white pigment-concentrating responses. A, B, responses to extract prepared by triturating the tissue directly in saline; C, D, responses to the ethanol-soluble fraction; E, F, responses to the 95% methanol:chloroform (2:1)-soluble fraction.

evoked by the ethanol-soluble and methanol:chloroform-soluble fractions of the horseshoe crab CNS would be compared with the responses to the saline extract to learn whether the chromatophorotropic material in the horseshoe crab CNS behaves in a similar manner to the melanin-dispersing material in the eyestalk of the fiddler crab. The extracts used in this particular set of experiments were prepared in a final concentration of 0.1 equivalent of the CNS of the horseshoe crab per dose

of 0.05 ml. The first extract was made by triturating three freshly dissected CNS in 1.5 ml saline which provided the desired final concentration. Before injection the extract was centrifuged for three minutes at 1815 × *g* and at 24° C. The ethanol-soluble fraction was obtained by extracting three freshly dissected CNS in

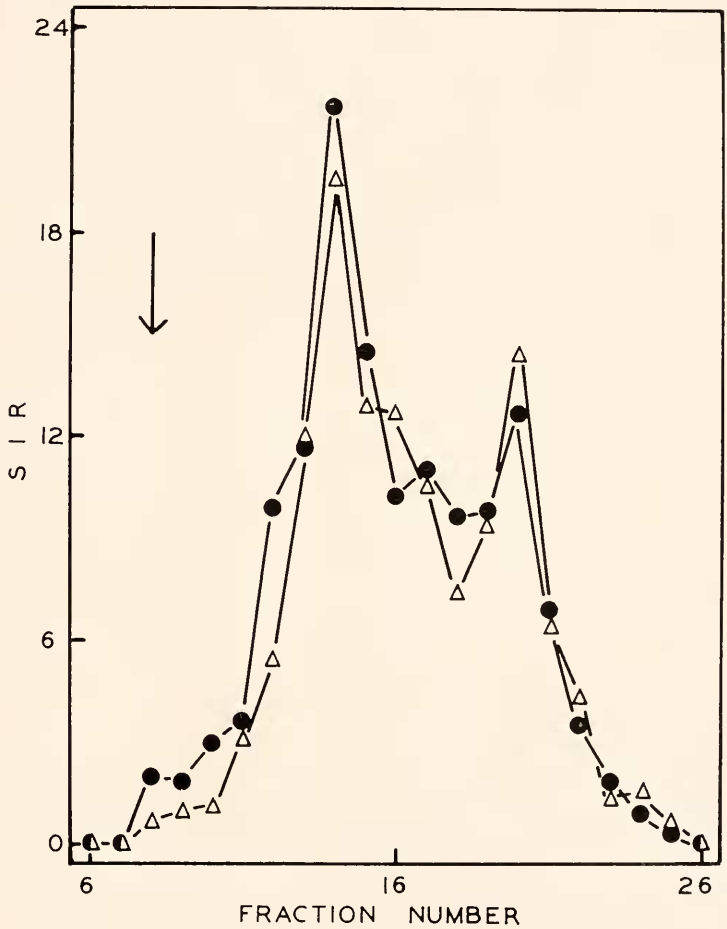

FIGURE 2. The melanin-dispersing (dots) and white pigment-concentrating (triangles) Standard Integrated Responses (SIR) of eyestalkless fiddler crabs evoked by the fractions obtained by passing an extract of the central nervous system of the horseshoe crab through a column of Bio-Gel P-6, arrow, void volume.

a total of 10 ml of 100% ethanol. This extract was then centrifuged in the same manner as was the saline extract and the clear supernatant was poured into a porcelain dish and allowed to evaporate on a heating table set at 35–40° C. When the fraction was dry the material was then suspended in 1.5 ml of saline to obtain the desired final concentration. The third extract was prepared in the same man-

ner as was the ethanol-soluble fraction, the sole difference being that 95% methanol: chloroform (2:1) was substituted for the 100% ethanol.

The averaged results of this group of experiments, performed twice, are presented as SIR's in Figure 1. It is evident that the CNS of the horseshoe crab possesses melanin-dispersing and white pigment-concentrating activities as first reported by Brown and Cunningham (1941). However, the extracts had neither a pigment-dispersing nor pigment-concentrating effect on the erythrophores of the fiddler crab. It is clear from examination of Figure 1 that not only did the ethanol-soluble and 95% methanol:chloroform (2:1)-soluble fractions of the horseshoe crab CNS evoke both melanin-dispersing and white pigment-concentrating responses but with each of the two fractions the melanin-dispersing and white pigment-concentrating responses were more than three times as large as the corresponding responses obtained with the extract prepared directly in saline. The larger SIR's seen with the ethanol-soluble and 95% methanol:chloroform (2:1)-soluble fractions were due to the fact that these two fractions evoked responses that had both larger amplitudes and longer durations than did the responses to the extract prepared directly in saline. The melanin-dispersing responses generated by the saline extract, the ethanol-soluble fraction and the 95% methanol:choroform (2:1)-soluble fractions showed peak chromatophore stages of 3.1, 4.5 and 4.7 respectively, and the responses lasted 5.5, 12.5 and 15.0 hours respectively. The corresponding values for the white pigment-concentrating responses where the initial stage of the pigment was 5 were chromatophore stages of 2.2, 1.3 and 1.1, and the responses lasted 6, 13 and 16 hours respectively. An hypothesis of Bartell, Rao and Fingerman (1971) which is based in part on the differences in the physical state of the material in saline, ethanol and methanol:chloroform extracts of eyestalks from the fiddler crab and which explains these differences in the SIR's will be discussed below.

Gel filtration of extracts of the central nervous system of the horseshoe crab and eyestalks of the fiddler crab

The objectives of this experiment were to attempt to separate the melanin-dispersing activity from the white pigment-concentrating activity in the horseshoe crab CNS by means of gel filtration and to compare the rates of flow (R_f) for these activities with that of the material extracted from the radial nerves of the sea star and the eyestalks of the fiddler crab. R_f is defined as the quotient of the fraction void volume/elution volume. In the first experiment of this series 10 CNS were triturated in 0.5 ml distilled water and centrifuged as described above for the saline extract. The supernatant was then applied to the top of a column of Bio-Gel P-6 whose dimensions were 57 × 0.9 cm and eluted with distilled water. The void volume was 16 ml and the flow rate was 38 ml/hr. Two ml fractions were collected. The averaged results from a total of four chromatographic separations are presented in Figure 2. It is evident from examination of this figure that the elution pattern of the melanin-dispersing activity was virtually the same as that of the white pigment-concentrating activity. The major peak for both activities occurred in fraction 14 and had an R_f of 0.57. The averaged data for both activities showed a secondary peak in fraction 20 with an R_f of 0.4. It should be noted,

however, that whereas the R_f 0.57 peak was a consistent feature in every experiment, the secondary peak was observed in only three of the four experiments. The secondary peak at R_f 0.4 may represent an additional chromatophorotropic substance or substances.

Another gel, Sephadex LH-20, was then tried in an effort to separate the melanin-dispersing and white pigment-concentrating activities. The gel was pre-

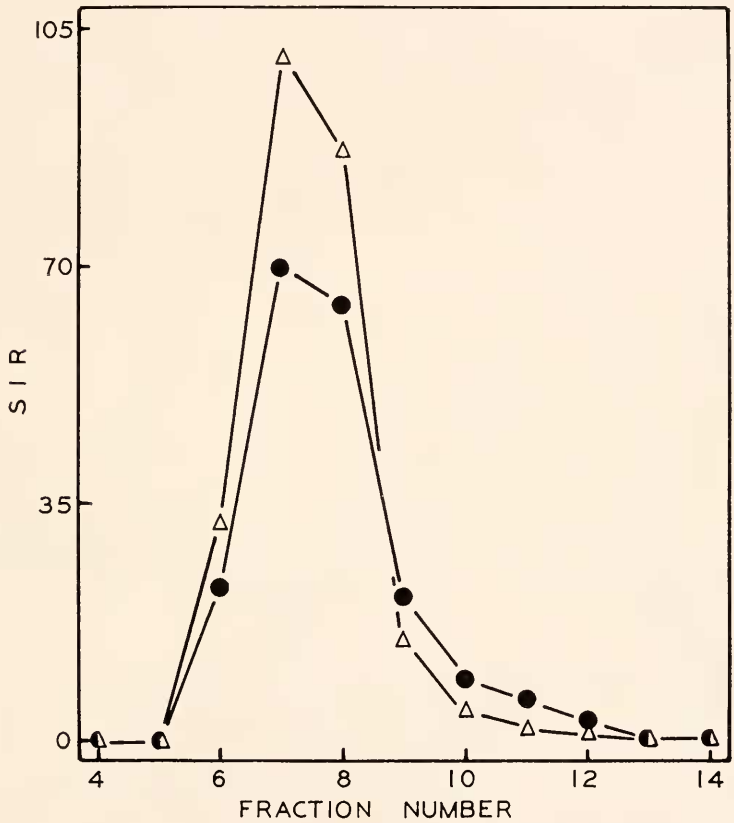

FIGURE 3. The melanin-dispersing (dots) and white pigment-concentrating (triangles) Standard Integrated Responses (SIR) of eyestalkless fiddler crabs evoked by passing the 95% methanol:chloroform (2:1)-soluble fraction extract of the central nervous system of the horseshoe crab through a column of Sephadex LH-20.

pared in 95% methanol:chloroform (2:1) which was also used as the elution medium and the solvent for extraction of the active material. Five freshly dissected CNS were extracted in 0.3 ml of the solvent, centrifuged as above and the supernatant was applied to the Sephadex LH-20 column whose dimensions were 28 × 0.7 cm. There is, of course, some dilution of the solvent because of the water in the tissue. The flow rate was 20 ml/hr and 1 ml fractions were collected. After the solvent had evaporated the residue from each fraction was suspended in

0.3 ml 100% saline and assayed. This experiment was performed twice and the averaged results are presented in Figure 3. It is clear from inspection of this figure that once again the melanin-dispersing and white pigment-concentrating activities did not separate from each other.

Although some chromatophorotropins from the fiddler crab have been analyzed previously by gel filtration (Fingerman and Couch, 1967; Fingerman and Rao, 1969a, 1969b), there has been no previous attempt to assay the several fractions

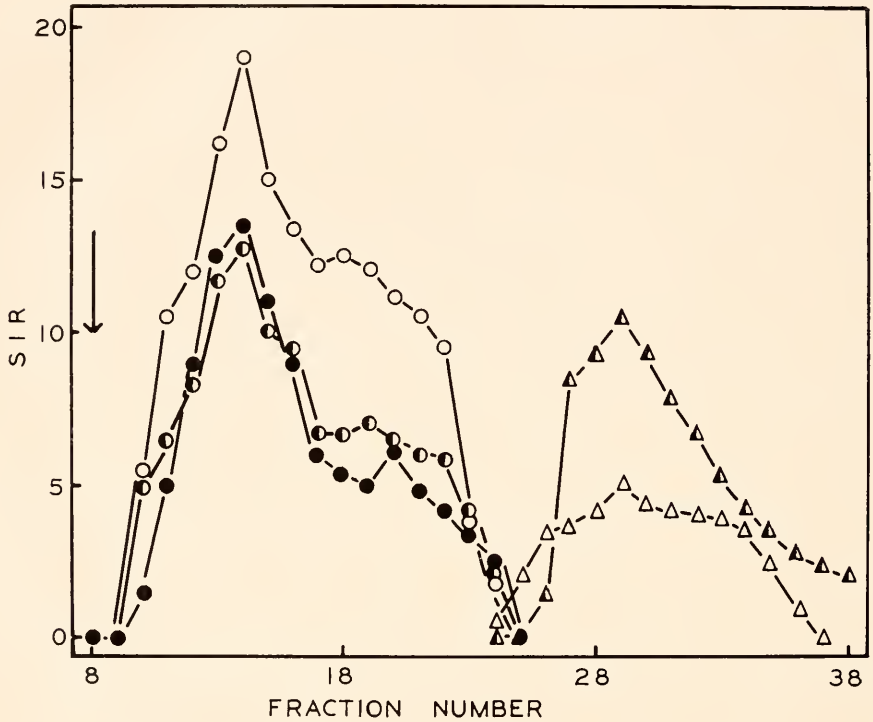

FIGURE 4. The Standard Integrated Responses (SIR) of fiddler crabs evoked by the fractions obtained by passing an extract of fiddler crab eyestalks through a column of Bio-Gel P-6; dots, melanin-dispersing responses; circles, white pigment-dispersing responses; half-filled circles, red pigment-dispersing responses; empty triangles, white pigment-concentrating responses; half-filled triangles, red pigment-concentrating responses, arrow, void volume.

simultaneously on the melanophores, leucophores and erythrophores. Consequently, definite conclusions concerning the elution patterns of the chromatophorotropins active on these three types of chromatophore were not possible. It was necessary to assay the fractions from the same chromatographic separation for several chromatophorotropic activities simultaneously if we were to attempt to determine the number of chromatophorotropins in the eyestalks of this fiddler crab. The resulting data could then be compared with those obtained (Fig. 2) after gel filtration of the extracts of the horseshoe crab CNS. The void volume of the column used to fractionate eyestalk extracts was 40 ml; the volume of each

fraction collected was 5 ml. A lyophilized distilled water extract of 300 eyestalks was dissolved in 1.5 ml distilled water and applied to a 71 × 1.5 cm column of Bio-Gel P-6 equilibrated and eluted with distilled water at a flow rate of 25 ml/hr. The several fractions generated not only melanin dispersion and white pigment concentration, but white pigment dispersion, red pigment dispersion and white pigment concentration as well. The averaged SIR's for the several chromatophorotropic responses are plotted in Figure 4 *versus* the fraction number. This experiment was performed twice. The pigment-concentrating material was sepa-

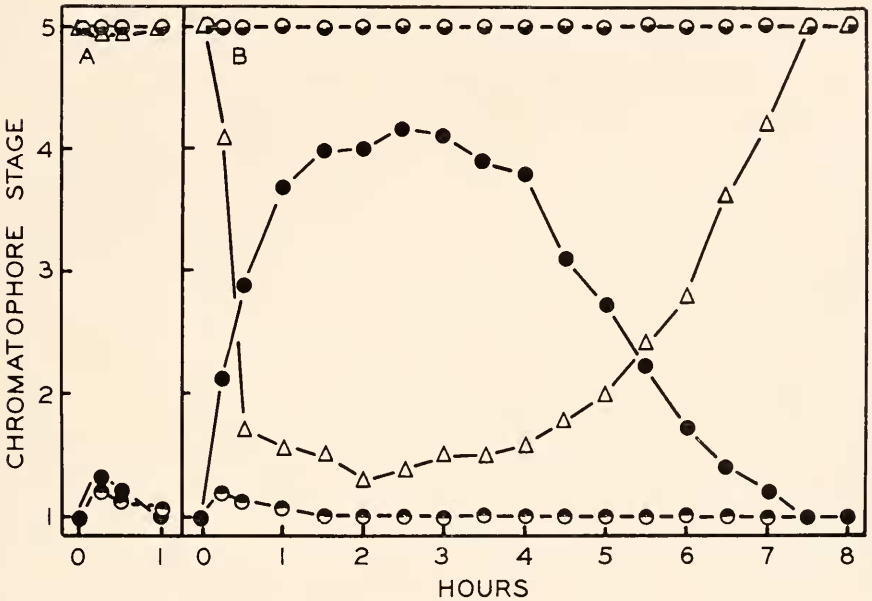

FIGURE 5. Relationships between chromatophore stage of eyestalkless fiddler crabs and time following injection of the (A) acetone-soluble and (B) acetone-insoluble fractions of the central nervous system of the horseshoe crab; dots, melanophores of crabs that received one of the fractions; triangles, white chromatophores of crabs that received one of the fractions; circles half-filled on top, melanophores of control crabs; circles half-filled on bottom, white chromatophores of control crabs.

rated by this gel from the pigment-dispersing material. The former had a peak at R_f 0.28 and the latter at 0.57. However, the three pigment-dispersing activities did not separate from each other. Likewise, the two pigment-concentrating activities did not separate from each other.

Test for acetone solubility of the chromatophorotropic material in the central nervous system of the horseshoe crab

The melanin-dispersing material from the fiddler crab, *Uca pugilator*, is insoluble in acetone (Abramowitz and Abramowitz, 1938) whereas the white pigment-concentrating substance of this crab is soluble in acetone (Rao, Fingerman and Bartell, 1967). Furthermore, the latter investigators were able to separate the

white pigment-dispersing and pigment-concentrating substances of this fiddler crab from each other by acetone extraction, the white pigment-dispersing substance being insoluble in acetone just as is the melanin-dispersing material. The present experiment was designed to determine whether the melanin-dispersing and white pigment-concentrating activities of the horseshoe crab CNS could be separated from each other by acetone extraction and if not then to determine whether the chromatophorotropic material from the horseshoe crab resembles the pigment-dispersers or the white pigment-concentrator of the fiddler crab with respect to acetone solubility.

The experiment, performed twice, was carried out in the following manner in a dehumidified room. Two CNS of the horseshoe crab were freeze-dried and then extracted three times with a total of 10 ml of acetone. The extract was centrifuged as above, the supernatant was poured into an evaporating dish and the acetone was allowed to evaporate on the warming stage. In similar fashion the insoluble material was dried. The dried acetone-soluble and acetone-insoluble fractions were then suspended in 1 ml of saline to provide a final concentration of 0.1 CNS equivalent per dose and assayed for melanin-dispersing and white pigment-concentrating activities. The average chromatophore stages are shown in Figure 5 where it can be seen that activity was present only in the acetone-insoluble fraction.

Discussion

These experiments have confirmed the observation of Brown and Cunningham (1941) that the CNS of the horseshoe crab evokes melanin-dispersion and white concentration in fiddler crabs. But whereas the fiddler crab, *Uca pugilator*, produces both red pigment-dispersing and pigment-concentrating substances (Brown, 1950), extracts of the horseshoe crab CNS have no effect on the erythrophores of this fiddler crab. The active material in the horseshoe crab CNS differs from that found in the radial nerve of the sea star by Rao and Fingerman (1970a) in the following ways. The latter material not only dispersed the melanin and concentrated the white pigment as did the horseshoe crab material, but in addition it concentrated the red chromatophoric pigment. Also, whereas the major peaks of activity for both melanin dispersion and white pigment concentration obtained after gel filtration of aqueous extracts of the horseshoe crab CNS both had an R_f of 0.57 on Bio-Gel P-6 (Fig. 2), the three activities from the sea star had an R_f of 0.39 which indicates that the chromatophorotropic material from the sea star has a smaller size. Although the material comprising the secondary peak, R_f 0.4, obtained with the horseshoe crab CNS extract on Bio-Gel P-6 is very close to that of the material in the sea star radial nerves (R_f 0.39), these materials nevertheless differ in their capacity to evoke a response of the erythrophores. The lack of response of the erythrophores could not have been due simply to a difference in the amount of horseshoe crab material injected as compared with that from the sea star. The SIR's of the melanophores and leucophores to the horseshoe crab material (Fig. 1A, B) were larger than the corresponding SIR's obtained with the fractions of the radial nerves (Rao and Fingerman, 1970a), and yet there was no response of the erythrophores to the horseshoe crab extract.

The R_f values for the pigment-dispersing and pigment-concentrating substances of the fiddler crab were 0.57 and 0.28, respectively (Fig. 4), indicating thereby that the pigment-concentrating activities are due to material of smaller size than are the pigment-dispersing activities. The sea star material with an R_f of 0.39 is apparently intermediate in size between them. On the other hand, the major peaks for both the melanin-dispersing and white pigment-concentrating activities of the horseshoe crab had the same R_f (0.57) as that of the pigment-dispersing activities of the fiddler crab. Whereas in the fiddler crab the pigment-dispersing and pigment-concentrating activities are clearly due to different molecules, both activities in the extracts from the CNS of the horseshoe crab might be due to the same molecule. If not the same molecule, then both activities are caused by different substances having similar elution patterns from Bio-Gel P-6. Just as with Bio-Gel P-6, chromatography on the gel Sephadex LH-20 failed to separate the two chromatophorotropic activities in the horseshoe crab CNS (Fig. 3). The possibility that pigment-dispersing and pigment-concentrating responses are both due to a single substance in the horseshoe crab CNS is not without precedent. The intermedins not only cause melanin dispersion in frog skin but concentration of the pigment in the guanophores as well (Bagnara, 1964).

The chromatophorotropic material in the horseshoe crab CNS is more closely related to the melanin-dispersing material of the fiddler crab than to the white pigment-concentrating hormone of the fiddler crab in three ways: (a) both the melanin-dispersing material of the fiddler crab and the material from the horseshoe crab that evokes melanin dispersion and white pigment concentration are insoluble in acetone (Abramowitz and Abramowitz, 1938; Fig. 5) but the white pigment-concentrating hormone of the fiddler crab is acetone-soluble (Rao, Fingerman and Bartell, 1967), (b) the melanin-dispersing material of the fiddler crab and the chromatophorotropic material from the horseshoe crab were eluted with an R_f of 0.57 (Figs. 2 and 4) from a column of Bio-Gel P-6 whereas the white pigment-concentrating hormone had an R_f of 0.28; and (c) whereas ethanol and methanol: chloroform can be used to obtain extracts of the horseshoe crab CNS (Fig. 1) and melanin-dispersing material from the eyestalks of the fiddler crab (Rao, Bartell and Fingerman, 1967; Bartell, Rao and Fingerman, 1971) which are more potent than extracts prepared directly in saline, the white pigment-concentrating hormone in these eyestalks does not behave in the same manner (Fingerman and Rao, 1969a).

As noted above, although the pigment-dispersing activities from the eyestalks of the fiddler crab could be separated from the pigment-concentrating activities, the three pigment-dispersing activities could not be separated from each other nor could the two pigment-concentrating activities be separated from each other on Bio-Gel P-6 (Fig. 4). Similarly, the pigment-dispersing activities in the eyestalks of the prawn, *Pandalus jordani*, could not be separated from each other by the chromatographic techniques that Kleinholz (1970) used. The respective pigment-dispersing and pigment-concentrating activities seen in Figure 4 are each caused by (a) a single molecule or (b) different substances having similar elution patterns from Bio-Gel P-6. Indirect evidence obtained with the fiddler crab, *Uca pugilator*, tends to favor the latter alternative. For example, in eyestalkless fiddler crabs of this species from Panacea, Florida, the melanin was maximally concentrated

whereas the white pigment showed all stages from maximal concentration to maximal dispersion (Rao, Fingerman and Bartell, 1967). If the chromatophores of a the fiddler crab were controlled by a single pigment-dispersing hormone and a single concentrator, then we would reasonably expect the pigments to be in lockstep, not having one fully dispersed while the other is fully concentrated. Furthermore, Rao, Fingerman and Bartell (1967) found that extracts of the circumesophageal connectives caused melanin dispersion in this crab, but did not disperse the white pigment. Fingerman and Couch (1967) using the gel Sephadex G-25 fractionated extracts of eyestalks from this fiddler crab and obtained some fractions which while evoking equal melanin-dispersing responses differed widely in their red pigment-dispersing activities. The differences were not due to antagonism by a red pigment-concentrating hormone. More recently, Rao and Fingerman (1970b) found that serotonin does not evoke melanin dispersion in this fiddler crab. But serotonin had an indirect effect on the erythrophores causing dispersion of the red pigment by stimulating release of a red pigment-dispersing substance. The occurrence of red pigment dispersion in the absence of melanin dispersion suggests that (a) different substances are involved or (b) melanin-dispersing substances also have red pigment-dispersing activity, but in addition there are discrete red pigment-dispersing substances.

Bartell, Rao and Fingerman (1971) have postulated that the high activities generated by ethanol-soluble and methanol:chloroform-soluble fractions of eyestalks from the fiddler crab are due to extraction of a micellar lipoprotein with a melanophorotropic polypeptide component. It was suggested that the lipoprotein micelles slowly release the polypetide, thereby accounting for the prolonged activity generated by these fractions. The same hypothesis can be applied to explain the prolonged responses obtained with the ethanol-soluble and 95% methanol:chloroform (2:1)-soluble fractions of the CNS of the horseshoe crab. Vigorous stirring and lyophilization of the ethanol-soluble fraction of the eyestalks dissociate the active polypeptide from the lipoprotein.

Summary and Conclusions

1. Extracts of the central nervous system of the horseshoe crab, *Limulus polyphemus*, were assayed for chromatophorotropic activity on the fiddler crab, *Uca pugilator*. The extracts caused pigment dispersion in the melanophores and pigment concentration in the leucophores but had no effect on the erythrophores.

2. The ethanol-soluble and 95% methanol:chloroform (2:1)-soluble fractions of the central nervous system from the horseshoe crab evoked melanin-dispersing and white pigment-concentrating responses which had larger amplitudes and longer durations than did the responses caused by extracts prepared directly in saline.

3. Neither gel filtration nor acetone fractionation was effective in separating the melanin-dispersing activity from the white pigment-concentrating activity in extracts of the central nervous system of the horseshoe crab. These responses appear to be caused by either the same molecule or by different substances having similar elution patterns from Bio-Gel P-6.

4. Extracts of the eyestalk of the fiddler crab were fractionated on Bio-Gel P-6. The melanin-dispersing, white pigment-dispersing and red pigment-dispersing ac-

tivities were eluted with an R_f of 0.57, the same value as that of the major peaks of melanin-dispersing and white pigment-concentrating activities from the central nervous system of the horseshoe crab. In contrast, the white pigment-concentrating and red pigment-concentrating activities of the fiddler crab separated from the pigment-dispersing activities, having been eluted later from the column of Bio-Gel P-6 with an R_f of 0.28.

5. The chromatophorotropic material in the central nervous system of the horseshoe crab is more closely related to the melanin-dispersing material of the fiddler crab than to the white pigment-concentrating hormone of the fiddler crab.

6. The chromatophorotropins in the central nervous system of the horseshoe crab and the radial nerves of the sea star differ from each other in their elution patterns from a column of Bio-Gel P-6 and in the chromatophore responses they evoke in the fiddler crab.

LITERATURE CITED

Abramowitz, A. A., and R. K. Abramowitz, 1938. On the specificity and related properties of the crustacean chromatophorotropic hormone. *Biol. Bull.*, **74**: 278–296.

Bagnara, J. T., 1964. Stimulation of melanophores and guanophores by melanophore-stimulating hormone peptides. *Gen. Comp. Endocrinol.*, **4**: 290–294.

Bartell, C. K., K. R. Rao and M. Fingerman, 1971. Comparison of the melanin-dispersing fractions in extracts prepared initially in ethanol, saline, or distilled water from eyestalks of the fiddler crab, *Uca pugilator*. *Comp. Biochem. Physiol.*, **38A**: 17–36.

Brown, F. A., Jr., 1950. Studies on the physiology of *Uca* red chromatophores. *Biol. Bull.*, **98**: 218–226.

Brown, F. A., Jr., and O. Cunningham, 1941. Upon the presence and distribution of a chromatophorotropic principle in the central nervous system of *Limulus*. *Biol. Bull.*, **81**: 80–95.

Carlson, S. P., 1935. The color changes in *Uca pugilator*. *Proc. Nat. Acad. Sci.*, **21**: 549–551.

Fingerman, M., and E. F. Couch, 1967. Differentiation of chromatophorotropins from the prawn, *Palaemonetes vulgaris*, and the fiddler crab, *Uca pugilator*. *J. Exp. Zool.*, **165**: 183–194.

Fingerman, M., and K. R. Rao, 1969a. A comparative study of leucophore-activating substances from the eyestalks of two crustaceans, *Palaemonetes vulgaris*, and *Uca pugilator*. *Biol. Bull.*, **136**: 200–215.

Fingerman, M., and K. R. Rao, 1969b. Physiology of the brown-black chromatophores of the stomatopod crustacean *Squilla empusa*. *Physiol. Zool.*, **42**: 138–147.

Fingerman, M., K. R. Rao and C. K. Bartell, 1967. A proposed uniform method of reporting response values for crustacean chromatophorotropins: the Standard Integrated Response. *Experientia*, **23**: 962.

Hogben, L., and D. Slome, 1931. The pigmentary effector system. VI. The dual character of endocrine co-ordination in amphibian colour change. *Proc. Roy. Soc. London, Series B*, **108**: 10–53.

Kleinholz, L. H., 1970. A progress report on the separation and purification of crustacean neurosecretory pigmentary-effector hormones. *Gen. Comp. Endocrinol.*, **14**: 578–588.

Pantin, C. F. A., The excitation of crustacean muscle. *J. Exp. Biol.*, **11**: 11–27.

Rao, K. R., and M. Fingerman, 1970a. Chromatophorotropic activity of extracts of radial nerves from the starfish, *Asterias amurensis*. *Z. Vergl. Physiol.*, **67**: 133–139.

Rao, K. R., and M. Fingerman, 1970b. Action of biogenic amines on crustacean chromatophores: 1. Differential effect of certain indolealkylamines on the melanophores of the crabs *Uca pugilator* and *Carcinus maenas*. *Experientia*, **26**: 383–384.

Rao, K. R., and M. Fingerman, 1970c. Action of biogenic amines on crustacean chromatophores, II. Analysis of the response of erythrophores in the fiddler crab, *Uca pugilator,* to indolealkylamines and an eyestalk hormone. *Comp. Gen. Pharmacol.,* **1**: 117–126.

Rao, K. R., C. K. Bartell and M. Fingerman, 1967. Relationship between the response of melanophores in the fiddler crab, *Uca pugilator,* and the concentration of eyestalk extract. *Z. Vergl. Physiol.,* **56**: 232–236.

Rao, K. R., C. K. Bartell and M. Fingerman, 1968. Solubility and stability properties of the melanin-dispersing substances in the eyestalks of the fiddler crab, *Uca pugilator. Z. Vergl. Physiol.,* **60**: 1–13.

Rao, K. R., M. Fingerman and C. K. Bartell, 1967. Physiology of the white chromatophores in the fiddler crab, *Uca pugilator. Biol. Bull.,* **133**: 606–617.

Sandeen, M. I., 1950. Chromatophorotropins in the central nervous system of *Uca pugilator,* with special reference to their origins and actions. *Physiol. Zool.,* **23**: 337–352.